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Two Coupled Oscillators: Simulations of the Circadian Pacemaker in Mammalian Activity Rhythms

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In the activity rhythms of captive small mammals a variety of features, most notably "splitting", suggest that two coupled oscillators may constitute the pacemaker system which underlies the rhythms. A phenomenological model proposed by Pittendrigh is developed and expanded here using an explicit quantitative structure. It is found that such a system can simulate several qualitative features in the experimental data: the interdependence of free-running period (τ) and activity time (α) with changing light intensity described in Aschoff's rule, after-effects on τ and α of prior conditions, and the occasional existence of two stable phase relationships, with different τ values for a given light intensity, as observed in "splitting". It is hoped that the model will suggest experiments aimed at the elucidation of the physiological basis of these phenomena.

1. Introduction

THE CASE FOR DAWN AND DUSK OSCILLATORS

In recent literature on circadian rhythms one repeatedly encounters the notion that there may be two separate oscillators governing circadian processes. The phenomena leading students of daily rhythms to this hypothesis are diverse. Two separate oscillators were originally proposed (Pittendrigh & Bruce, 1959) to account for transient resetting patterns in the rhythm of pupal eclosion in fruit-flies (*Drosophila pseudoobscura*). The hypothesis of a two-oscillator system has further been raised to account for such diverse phenomena as photoperiodic time measurement (Pittendrigh, 1960, 1972; Tyschenko, 1966), nocturnal migratory restlessness in birds (Gwinner, in Aschoff, 1967), seasonal shifts from nocturnal to diurnal activity and vice versa (Eriksson, 1973), "splitting" of circadian rhythms in constant conditions (Pittendrigh, 1960, 1974; Hoffmann, 1971), and changes in activity time with varying photoperiod (Pittendrigh, 1974; Daan &

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Aschoff, 1975). Various properties of insect eclosion rhythms (Engelmann, 1966) and of nyctinastic leaf movements (Engelmann & Honegger, 1967; Hoshizaki, Brest & Hamner, 1969, 1974) in different light-dark cycles have been interpreted as suggesting separate oscillating systems, one coupled to dawn and the other to dusk.

For many of these phenomena, there is no direct evidence of two distinct oscillators. Most of the behaviour of the rhythms observed may also be interpreted as complex responses of a single basic oscillator. For example while Saunders' (1973) demonstration of thermo-periodic induction of diapause in *Nasonia vitripennis* supports a model of "internal coincidence" (Pittendrigh, 1972) involving two oscillators, his results are open to other explanations. The phenomenon could result from a single oscillator with a specific phase which is both light- and temperature-sensitive, in agreement with Bünning's (1936) classic hypothesis.

The most compelling evidence for a two oscillator system in vertebrates is the occurrence of "splitting" of free-running activity rhythms into two distinct components. These typically run for a number of cycles with different frequencies before locking on to a stable condition with the two components in 180° antiphase to one another (Pittendrigh, 1960). While splitting occurs frequently in the golden hamster exposed to constant bright light (Pittendrigh, 1967, 1974; Pittendrigh & Daan, 1976*c*), occasionally in other animals (Kramm, 1971; Pohl, 1972), and in birds (Pohl, 1971; Gwinner, 1974), it has been most consistently demonstrated by Hoffmann (1969, 1971) in the tree shrew (*Tupaia belangeri*) subjected to constant dim illumination. In lizards, a dissociation of activity rhythms into two components with different frequencies has recently been reported by Underwood (1977). We fail to see how a single oscillator could simultaneously produce two sharply defined non-harmonic frequencies.

Splitting occurs exclusively in constant conditions. In conditions of entrainment a bimodal pattern of activity is fairly common in both nocturnal and diurnal species (Aschoff, 1962). Only in this case there are some indications for dawn- and dusk-coupling of two separate components. In the deer-mouse *Peromyscus leucopus* the two peaks of nocturnal activity have been observed to run with different frequencies in constant darkness preceding entrainment (Pittendrigh & Daan, 1976*c*, Fig. 7). In other species two peaks are known to lock on to dawn and dusk with changing daylength (Aschoff, 1962; Eriksson, 1973). Such features are easily accommodated by the assumption that two oscillators responding differently to the entraining signals of dawn and dusk are involved. This would give rise to observed changes in activity time (α) with changing photoperiod (Pittendrigh, 1974; Daan & Aschoff, 1975). While the dawn- and dusk-oscillator concept thus

has several attractive features, it is unlikely that more definitive evidence will come from studies on activity alone. Such evidence might result from studies in which correlates of the components were identified physiologically and then experimentally separated or uncoupled.

Evidently, a model for circadian pacemakers consisting of two coupled oscillators should initially clarify the splitting phenomenon and its empirical regularities. Such a model has been proposed in qualitative terms by Pittendrigh (1974) and Pittendrigh & Daan (1976*c*). The basic propositions of this model are the following:

The pacemaker responsible for circadian activity rhythms in rodents consists of two separate oscillators, E and M, coupled to each other in a stable phase relationship (ϕ_{EM}). ϕ_{EM} depends on the spontaneous frequencies, i.e. the frequencies that would be displayed in the absence of coupling, of E and M, which respond differentially to light intensity. In turn the period of the coupled system (τ) varies with ϕ_{EM}, which also determines the activity time (α) since onset of activity (*E*vening peak in nocturnal animals) and end of activity (*M*orning peak) are each under control of one of the oscillators. In certain light conditions the oscillators can assume either of two stable phase relationships. The system tends to preserve any established ϕ_{EM}. These propositions were claimed to account for the patterns of splitting, as well as for other empirical generalizations, especially the history dependence of τ and α known from "after-effects", the interdependence of τ and α described in "Aschoff's rule", and the general bimodality of daily activity patterns (Pittendrigh & Daan, 1976*c*). A mathematical treatment makes explicit the structure of a self-consistent model and reveals the essential properties required to simulate the phenomenology.

2. Assumptions and Basic Principles

Most models of circadian oscillators have been abstract, in the sense that they include parameters not definable in terms of concrete physiological or biochemical processes (however, see Cummings, 1975). This feature makes them difficult to test and limits their heuristic or predictive value. In the absence of information regarding the state variables relevant to circadian pacemakers, we also employ an abstract model, using it more to display and organize consequences of a general two-oscillator scheme than to yield mechanistic predictions. We have therefore chosen to use two component oscillators as non-specific as possible, and thus have rejected differential equation models, e.g. of the van der Pol type. The terms in such equations lack obvious physiological correlates and their analysis contributes to computer time more than to better understanding of biological reality. The

algorithm used is based on two oscillators defined entirely in terms of their time course (period length, phase and phase shifts). Their interaction is by instantaneous resets similar to Pavlidis' (1973, p. 179) analysis of firefly synchronization.

Each of our two oscillators (designated E and M) is fully determined by a series of events E and M, which recur with constant intervals τ_E and τ_M, respectively, when the oscillators are uncoupled. E_i, M_i, $i = 1, 2, \ldots$, denote the times of occurrence of E and M. Of the parameters E_1, M_1, τ_E and τ_M, which fully determine the time course of all events, the first two, defining initial conditions, are relatively unimportant, and the last two can be reduced to one variable, the *detuning* $D = \tau_E - \tau_M$, by fixing $\tau_E = 24 + \frac{1}{2}D$ h and $\tau_M = 24 - \frac{1}{2}D$ h. Thus, we have assumed that the natural periods of the two oscillators vary symmetrically around a fixed value, which was set at 24 h for the purpose of graphic display in the standard experimental format of actograms. The two-oscillator hypothesis of Pittendrigh (1974) and Pittendrigh & Daan (1976*c*) assumes that the natural period (τ_E) of one oscillator increases and the natural period (τ_M) of the other decreases with increasing intensity of constant illumination (*LL*). This assumption can be translated in terms of our model by regarding D as a positive monotonic function of light intensity.

We have made no assumption on the mechanism or on the amplitude of the oscillations leading to the recurrence of the events E and M, and have only assumed that these events recur periodically. Unlike phase and frequency, amplitude of a pacemaking oscillation bears no necessary relation to the properties of the rhythm it drives. The events can be thought of as triggers leading to a period of activity. In the displays based on some of the computer simulations (Figs 3–6), we have associated 5 h of activity with each of the events E and M.

Clearly, for any $D \neq 0$, the events E and M recur with different frequencies and continuously drift relative to each other. Coupling is achieved by resetting the E-oscillator by Δe h at the time of each event M and by resetting the M-oscillator by Δm h at the time of each event E (Fig. 1).

Let E_{j-1} be the time of the E-event preceding M_i. Let e_j be the interval $E_j - M_i$; and m_i the interval $M_i - E_{j-1}$. The recursive coupling equations used then are:

$$e_j = \tau_E - m_i + A_E \sin \frac{2\pi}{\tau_E}(m_i + \phi_E),$$

and

$$m_i = \tau_M - e_{j-1} + A_M \sin \frac{2\pi}{\tau_M}(e_{j-1} + \phi_M).$$

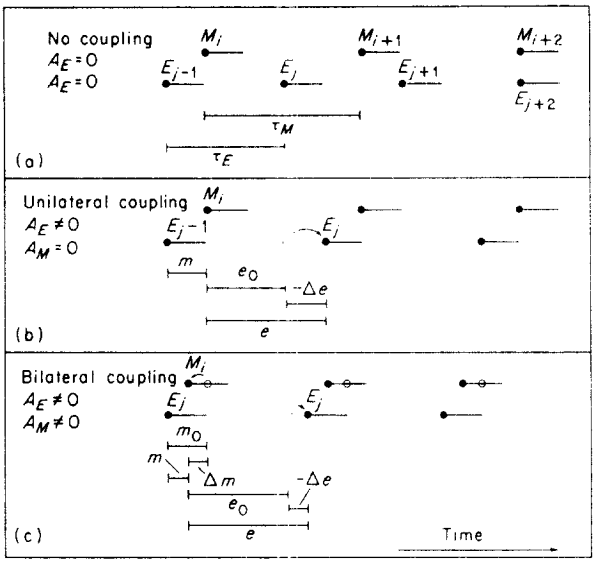

FIG. 1. Coupling procedure and definitions. Dots mark the time of periodic events M and E produced by the two oscillators. In the absence of coupling the events recur with their spontaneous frequency (periods τ_M and τ_E). Unilateral coupling is achieved by phase shifting one oscillator (E) by an amount Δe every time when an event M_i occurs. Δe is a function(phase response curve) of e_0. Negative Δe are interpreted as delays, positive Δe as advances. The coupled system assumes the period τ_M of the driving oscillator. Bilateral coupling occurs when both A_E and A_M are non-zero and phase shifts Δm and Δe are induced in oscillators M and E at times E_j and M_i, respectively. The period of the coupled system differs from both τ_M and τ_E. For further explanation see text.

A_E and A_M are arbitrary parameters, determining the strength of coupling in both directions; ϕ_E and ϕ_M are arbitrary parameters, defining the phase of the reset curves relative to the E and M events. Sine wave reset curves were chosen as a simple form of a two-sign phase response curve, such that each of the oscillators can assume both a shorter and a longer period than its natural period when under the influence of the other.

Figure 1 shows the effects of coupling on the sequence of E and M events, using the short notations:

$$e_0 = \tau_E - m_i; \quad m_0 = \tau_M - e_{j-1};$$

$$\Delta e = -A_E \sin \frac{2\pi}{\tau_E} (m_i + \phi_E)$$

and

$$\Delta m = -A_M \sin \frac{2\pi}{\tau_E} (e_{j-1} + \phi_M).$$

If $A_M = 0$, coupling is unilateral, and the system in synchrony always assumes the frequency of oscillator M [Fig. 1(b)].

The two oscillators are in synchrony if successive e_j's and m_i's are the same, these being the solutions of the equilibrium equations:

$$e = f_E(m) = \tau_E - m + A_E \sin \frac{2\pi}{\tau_E}(m + \phi_E),$$

$$m = f_M(e) = \tau_M - e + A_M \sin \frac{2\pi}{\tau_M}(e + \phi_M).$$

A graphic analysis (Fig. 2) aids in understanding the basic properties of the system. The functions $f_E(m)$ and $f_M(e)$ are plotted in a plane (m, e). Any point shared by the two functions is an equilibrium solution. For such a solution (m^*, e^*), the period of the coupled system is $\tau = m^* + e^*$.

When there is *no coupling* [$A_E = A_M = 0$; Fig. 2(a)], then the functions $f_E(m) = \tau_E - m$ and $f_M(e) = \tau_M - e$ describe parallel lines, having no solution, or a single line (when $\tau_E = \tau_M$). In the first case there is no synchrony, in the latter case synchrony occurs at any (e, m).

With *unilateral coupling*, e.g. with only the E-oscillator reset by M-events [$A_M = 0$, $A_E \neq 0$; Fig. 2(b)], $f_E(m)$ is curved and may have two points in common with the straight line $f_M(e)$. A set of recurring interval lengths m^* and e^* corresponds with each of the two points. In both cases the intervals add up to τ_M: with synchrony caused by unilateral coupling, the system assumes the period of the "driving" oscillator (M).

With *bilateral coupling*, both $f_E(m)$ and $f_M(e)$ are curved. By varying the system parameters any number of equilibrium solutions from zero to four can be obtained [two in Fig. 2(c), four in Fig. 2(d)].

Some of the equilibria are stable and others are unstable. Stable and unstable equilibria can easily be distinguished by comparing the slopes of the two curves at the intersection (May, 1975). The stability criterion is:

$$|f'_E(m^*) \cdot f'_M(e^*)| < 1,$$

as a result of perturbation analysis applied to m^* and e^*. The slopes of the curves in the point (m^*, e^*) are $f'_E(m^*)$ and $1/[f'_M(e^*)]$, respectively. Hence in a stable equilibrium the curve of $f_E(m)$ is closer to horizontal than $f_M(e)$. This criterion is of course easily detected graphically.

As Fig. 2(b)–(d) illustrate, stable and unstable solutions alternate. Thus with those particular sets of parameter values leading to four solutions of $f_E(m)$ and $f_M(e)$, there are two stable and two unstable solutions [Fig. 2(d)]. The stable equilibria have e^*-values differing by about $\frac{1}{2}\tau$, i.e. $c.$ 180°. That is, they are characterized by opposite phase relationships between the

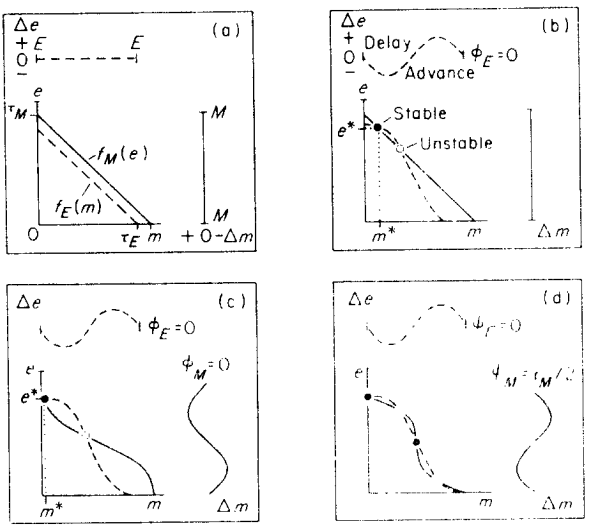

FIG. 2. Graphic solutions of the equations $e = \tau_E - m - \Delta e$ and $m = \tau_M - e - \Delta m$. Each panel contains three graphs: Δe as a function of m in the upper left corner; Δm as a function of e in the lower right corner and both $f_M(e)$ and $f_E(m)$ in the lower left corner.

(a) *No coupling* ($A_M = 0$, $A_E = 0$). The functions $f_E(m)$ and $f_M(e)$ have no point in common: there is no synchrony, unless τ_E equals τ_M, in which case they have every point in common and any phase relationship is constant.

(b) *Unilateral coupling* ($A_M = 0$; $A_E \neq 0$). $f_E(m)$ and $f_M(e)$ have 0, 1, or 2 points in common. With two solutions, one is stable (m^*, e^*, solid symbol), the other unstable (open symbol). The two oscillators attain synchrony with the events M occurring m^* after the events E, and with system period $\tau = \tau_M = m^* + e^*$.

(c) *Bilateral coupling* ($A_M \neq 0$; $A_E \neq 0$). $f_E(m)$ and $f_M(e)$ have 0, 1, 2, 3, or 4 points in common. With two solutions, there is again one stable (m^*, e^*; solid symbol) and one unstable (open symbol). In synchrony, the two oscillators have a period $\tau = m^* + e^*$, which is in this case intermediate between τ_M and τ_E.

(d) *Bilateral coupling* ($A_M \neq 0$; $A_E \neq 0$): by changing ϕ_M from 0–$\tau_M/2$ h, the number of solutions is raised from 2–4. There are two stable and two unstable solutions. The stable solutions have phase angle differences about 180° apart.

component oscillators. It can be shown that two stable equilibria occur if $\tau_E - \tau_M = 0$, $A_E - A_M = 0$ and $\phi_E + \phi_M = \frac{1}{2}\tau_E$ or $\frac{3}{2}\tau_E$. Hence two identical oscillators ($\tau_E = \tau_M$, $A_E = A_M$) have two stable phase relationships when $\phi_E = \phi_M = \frac{1}{4}\tau_E$ or $\frac{3}{4}\tau_E$. The stability of the equilibria implies that small departures from these conditions will not remove either equilibrium. Larger changes may remove one equilibrium and force the system into the other. Apparently, this can be achieved by *differential* changes in τ_E and τ_M or in A_E and A_M or by *identical* changes in ϕ_E and ϕ_M. We emphasize the occurrence of two stable solutions, since they have biological relevance for the process of splitting, to which we return in section 3.

3. Simulations

(A) ASCHOFF'S RULE, AFTER-EFFECTS AND SPLITTING

The analysis is concerned mainly with demonstrating that dynamics similar to those observed experimentally are possible. Since the parameters have no known physiological counterpart, there is little use for a systematic exploration of the boundaries of the arrays of parameter values within which the behaviour of the model qualitatively matches observed behaviour of real pacemakers. There are of course some obvious constraints. For instance, in order to retain synchrony, it is necessary that the two oscillators be coupled with enough strength to overcome their detuning. The faster oscillator, e.g. oscillator E, can be slowed down at the most to a period of τ_E plus the maximum of the resetting curve for E, hence to $\tau_E + A_E$. For synchrony, this obviously should be no less than the minimum period of oscillator M:

$$\tau_E + A_E \geq \tau_M - A_M,$$

or

$$A_E + A_M \geq -D.$$

Similarly, if M is the fastest oscillator:

$$A_E + A_M \geq D.$$

Thus stable coupling requires as a minimum condition that:

$$A_E + A_M \geq |D|.$$

Still other criteria should be met to obtain at least one stable equilibrium.

Figure 3 shows an actogram obtained by computer simulation, with parameter values selected to simulate the free-running activity rhythm in a nocturnal rodent. Three values of D are used to simulate the effect of different light intensities. A low value ($D = -0{\cdot}15$), meaning that oscillator E by itself is 0·15 h faster than oscillator M, is taken to correspond with low light intensity *LL*. τ of the coupled system is shorter than 24 h. With D increasing in two steps, τ increases, and when D is set again at $D = -0{\cdot}15$, τ returns to its original value. The system is made, by choice of parameters, to follow "Aschoff's rule" for nocturnal animals. There are two important features in the simulation. First, with increasing D, the phase relationship between the two oscillators changes: the intervals m are reduced, the intervals e are increased. This is reflected in a decrease in the total activity time (α). Second, and more importantly, the changes from one condition to the next are gradual instead of abrupt. Following the step from $D = -0{\cdot}15$ to $D = -0{\cdot}05$, it takes the system some 30 cycles to revert from a period shorter than 24 h to a period longer than 24 h. And when D is abruptly reduced from $+0{\cdot}05$ to $-0{\cdot}15$, the system initially continues with $\tau \geq 24$ h

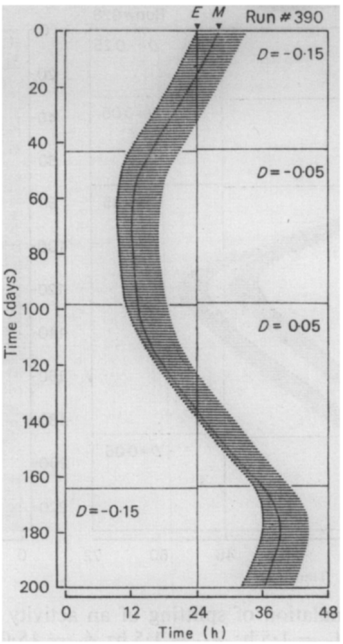

FIG. 3. Computer simulation of a circadian activity rhythm using the two-oscillator algorithm. The time at which events E and M occur is indicated by dots. Starting with each event a period of 5 h of "activity" is indicated by a horizontal line. For clarity, the horizontal time axis is continued to the right across the 24 h limit ("double plot"; "triple plot" in Fig. 4). Parameter values in this simulation are: $A_E = 1{\cdot}0$ h; $A_M = 1{\cdot}0$ h; $\phi_E = 15{\cdot}00$ h; $\phi_M = 21{\cdot}25$ h. Values of $D = \tau_E - \tau_M$ were changed thrice during the run after steady-state synchrony was attained. Notice the increase in system period (τ), the decrease in activity time (α) with increasing D, and the initial after-effect following changes in D.

before gradually returning to the steady state τ belonging to $D = -0{\cdot}15$. Also, the change in activity time is gradual. Clearly there is a *history dependence* to the instantaneous values of τ and α measurable during each cycle. The phase relationship between the oscillators changes gradually, and τ is apparently determined by this phase relationship, while all system parameters remain unchanged. The history dependence closely parallels that found experimentally (Pittendrigh & Daan, 1976*a*) in the case of after-effects of various constant light intensities.

The same features are seen in Fig. 4(a), using slightly different parameter values. α decreases and τ increases gradually when D is raised from $-0{\cdot}25$ to $+0{\cdot}05$. By raising D further to 0·35, initially a further increase in τ and decrease in α are observed. However, after some 25 cycles a dramatic change

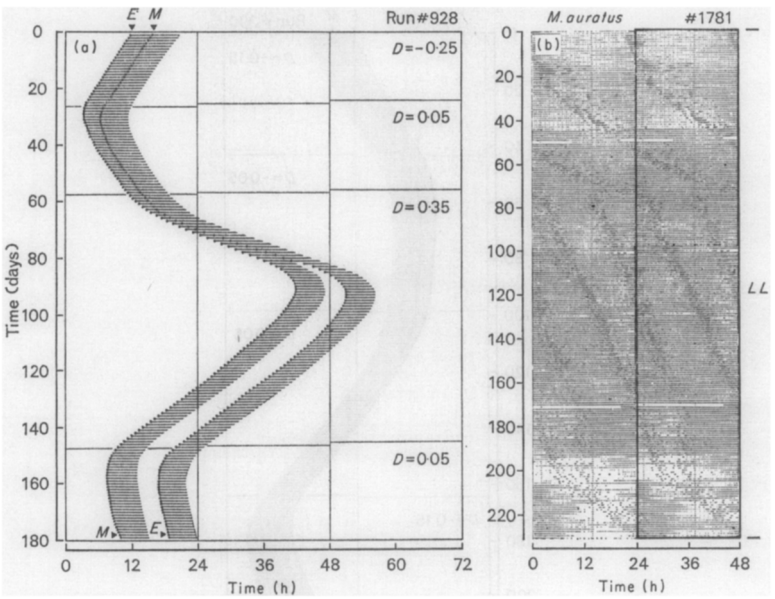

FIG. 4. (a) Computer simulation of splitting of an activity rhythm. Conventions as in Fig. 3. Parameter values: $A_E = 1{\cdot}5$ h; $A_M = 1{\cdot}5$ h; $\phi_E = 15{\cdot}0$; $\phi_M = 21{\cdot}25$ h. Notice the stepwise increase in τ with detuning D, the time lag following the increase in D to 0·35 until *splitting* into two components occurs, the change in τ associated with splitting, and the existence of two stable phase relationships between the oscillators for $D = 0{\cdot}05$.

(b) Spontaneous splitting and refusing in a free-running circadian activity rhythm in a golden hamster (*Mesocricetus auratus*) in constant illumination (from Pittendrigh & Daan, 1976*c*, Fig. 5). Notice the changes in τ associated with splitting and refusion.

occurs, as though spontaneously. The two oscillators move into a wholly different phase relationship with each other, nearly 180° different from where they started. What was one solid band of activity initially splits into two components. Simultaneously, τ of the system ostensibly shortens. When D is set back again to 0·05, the system remains in the new, split condition, and τ lengthens. Apparently, for $D = 0{\cdot}05$, in combination with this particular set of parameter values, there are two stable equilibria, as was postulated earlier in this section there should be for some sets. Which of the two equilibria is actually realized depends on the phase relationship between the two oscillators that was present at the moment when D was set at 0·05. Clearly, this is a *hysteresis* phenomenon, which matches the splitting behaviour as described in *Tupaja belangeri* by Hoffmann (1971). At intermediate light intensities (*c.* 1–100 lux), *Tupaja* rhythms can be either split or unsplit, depending on whether they had been forced into the unsplit condition by

high intensity *LL*, or in the split condition by low intensity *LL*. The spontaneous change in τ associated with splitting is qualitatively the same as that observed by Pittendrigh & Daan (1976*c*) in experiments in hamsters [Fig. 4(b)]. Another aspect of the simulation worth noticing is that in the two modes of coupling, the dependence of τ on D is clearly different. The transfer from $D = 0{\cdot}05$ to $D = 0{\cdot}35$ in the unsplit condition induces an increase in τ; the transfer back to $D = 0{\cdot}05$ in the split condition causes τ again to increase. There are no empirical data on this dependence in hamsters. However, in *Tupaja* Hoffmann (pers. comm.) has observed a difference in the dependence of τ on light intensity in the split and unsplit conditions.

"Splitting" and "refusion" of the two components of the system does not necessarily involve changes in the detuning (D) of the two oscillators, as suggested originally (Pittendrigh, 1974; Pittendrigh & Daan, 1976*c*). In the simulations shown in Fig. 5, detuning was kept constant ($D = 0$) throughout the run, and the relative strength of coupling in both directions was varied

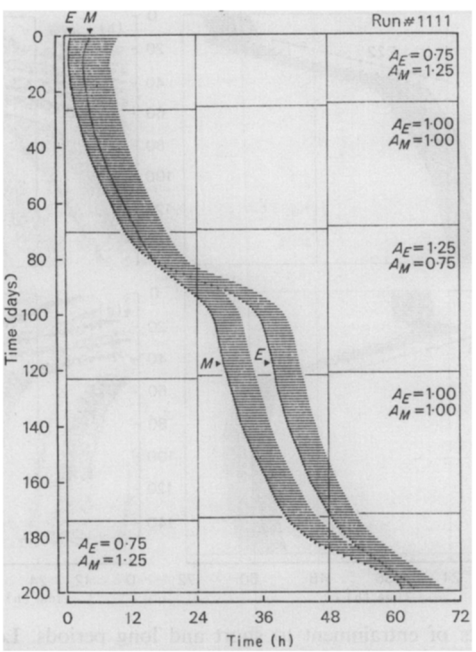

FIG. 5. Computer simulation of splitting and refusion of two components of a circadian activity rhythm. Conventions as in Fig. 3. Parameter values: $D = 0$, $\phi_E = 15{\cdot}00$, $\phi_M = 21{\cdot}25$, A_E and A_M as indicated. Notice the split developing around "day" 90, after raising A_E to 1·25 and lowering A_M to 0·75. The split results in a significant shortening of τ, refusion after day 180 in lengthening of τ. Notice further the existence of two stable phase relationships, 180° apart, when $A_E = 1{\cdot}00$ and $A_M = 1{\cdot}00$ (days 26–70 and 123–174).

by changing A_E and A_M. The period of the coupled system changes with coupling strength and hysteresis is again found as in Fig. 4; splitting occurs when $A_E = 1{\cdot}25$ and $A_M = 0{\cdot}75$ and refusion not until $A_E = 0{\cdot}75$ and $A_M = 1{\cdot}25$. Thus our model does not specify whether the effect of light in free-running conditions is on the natural frequencies of the two oscillators, as originally presumed, or on their coupling.

In order to display the consequences of entrainment for the subsequent behaviour of the system in free-running conditions, we have extended the model with a third oscillator. This oscillator consists of a series of events (zeitgeber signals), recurring at fixed intervals (T), each of which resets both the E- and M-oscillators. Thus, each zeitgeber-event at time Z_k resets the E-oscillator by an amount of:

$$A_{ZE} \sin \frac{2\pi}{\tau_E} (E_i - Z_k - \phi_{ZE}) \text{ h},$$

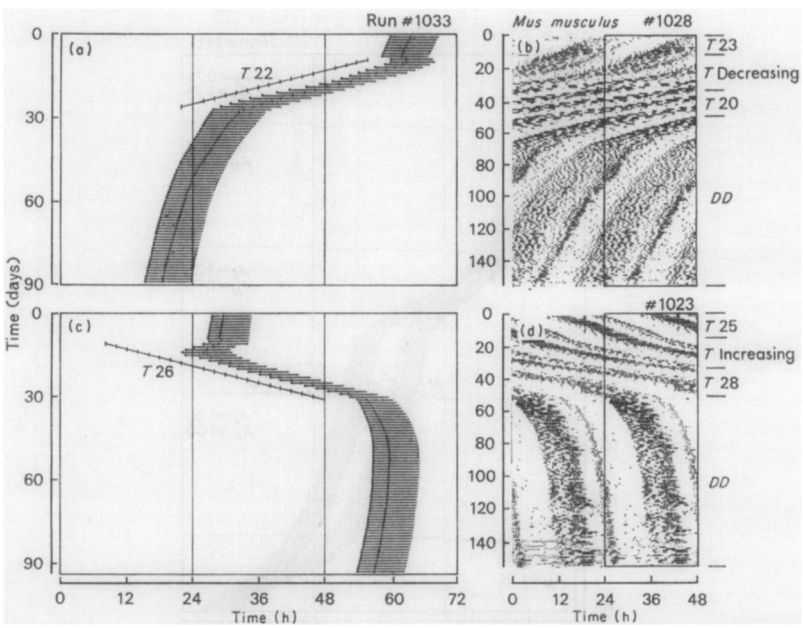

FIG. 6. After-effects of entrainment to short and long periods. Left panel: computer simulation. Entrainment is achieved by resetting the two oscillators every 22 h (T 22) or every 26 h (T 26) as dictated by sine-wave phase response curves. Other conventions and parameter values as in Fig. 3. $D = 0{\cdot}10$ h. Following entrainment by T 22, the initial period of the system was 23.5 h, following entrainment by T 26, the initial period was 24·4 h. Steady state τ at the end of the free-run was 23·9 h in both cases. Right panel: Short τ after T 20 and long τ after T 28 exhibited by two mice (*Mus musculus*) in constant darkness. Data from Pittendrigh & Daan (1976*a*, Fig. 7).

and the M-oscillator by

$$A_{ZM} \sin \frac{2\pi}{\tau_M} (M_j - Z_k - \phi_{ZM}) \text{ h},$$

where E_i and M_j are the times of the E- and M-events following Z_k. A_{ZE}, A_{ZM}, φ_{ZE} and φ_{ZM} are arbitrary parameters defining amplitude and phase of the phase response curves of the oscillators with respect to the zeitgeber.

The system was entrained for a number of cycles by zeitgebers with period $T = 22$ h [Fig. 6(a)] and with $T = 26$ h [Fig. 6(c)]. In the entrained steady states different phase relationships between E and M are established. It is these phase relationships which function as the initial conditions when the series of signals is interrupted, and hence determine the initial period τ in the subsequent free-run. After entrainment by $T = 22$, the system initially has a period shorter than the τ to which it gradually reverts in the course of many cycles. After $T = 26$, the initial period is longer than 24 h and only gradually drifts back to the same value smaller than 24 h, as is eventually attained after $T = 22$. Thus the system of two coupled oscillators offers a straightforward explanation of the phenomenon of after-effects of entrainment with different zeitgeber periods, observed in experiments [Pittendrigh & Daan, 1976*a*; and see Fig. 6(b), (d)].

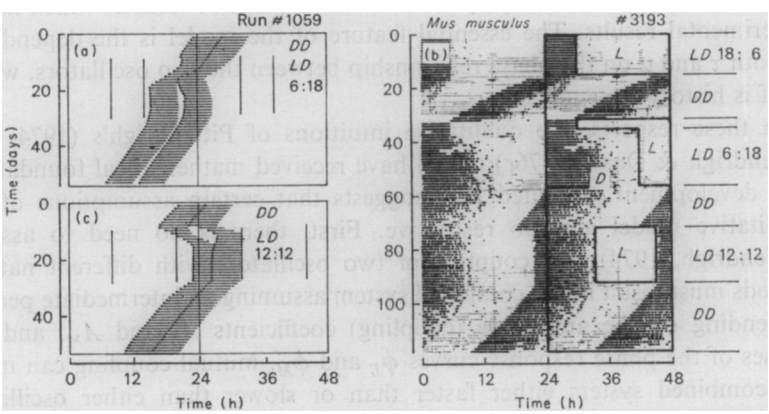

FIG. 7. After effects of entrainment by long and short photoperiods in $T = 24$ h.

(a), (c) Computer simulations. Parameter values: $A_E = 1{\cdot}5$ h; $A_M = 1{\cdot}0$ h; $\phi_E = 6{\cdot}0$ h; $\phi_M = 4{\cdot}5$ h. During entrainment, the two oscillators are reset twice every 24 h, with intervals of 6 and 18 h between the resets (*LD* 6:18) and with intervals of 12 and 12 h (*LD* 12:12). Upon termination of the resetting procedure, the period (τ) is initially shorter after 12:12 than after 6:18.

(b) Actogram of a mouse (*Mus musculus*) exposed successively to various photoperiods (*LD* 18:6; *LD* 6:18 and *LD* 12:12) with periods of constant darkness (*DD*) in between. In *DD*, τ was longer after the short (*LD* 6:18, 12:12) than after the long photoperiods (*LD* 18:6). Data from Pittendrigh & Daan (1976*a*, Fig. 14).

Similarly, after entrainment by simulated "skeleton photoperiods", i.e. by two resetting signals every 24 h, spaced L and $(24-L)$ h apart, the initial period of the coupled system is different for different L (Fig. 7). Again there are after-effects due to the difference in phase relationship between the constituent oscillators established by different photoperiods. τ is initially longer after $L = 6$ than after $L = 12$, just as observed in experiments with several species of nocturnal rodents [Pittendrigh & Daan, 1976*a*; and see Fig. 7(b)].

4. Discussion

Computations with a simple algorithm portraying the behaviour of two coupled oscillators show that by choice of parameter values such a system can be made to display a number of features characteristic of circadian activity rhythms in constant conditions. The model offers an account of the inter-relationships between free-running period (τ) and activity time (α) described in Aschoff's rule, which is one of the major generalizations on circadian rhythms (Daan & Pittendrigh, 1976). For some selected sets of parameter values two stable phase relationships occur, characterized by different τ, and by hysteresis in passage from one state to the other. Furthermore the computations display after-effects of prior conditions which mimic experimental results. The essential feature of the model is the dependence of both τ and α on the phase relationship between the two oscillators, which itself is history-dependent.

In these respects, the qualitative intuitions of Pittendrigh's (1974) and Pittendrigh & Daan (1976*c*) model have received mathematical foundation. The development presented here suggests that certain assumptions of the qualitative model are too restrictive. First, there is no need to assume (Pittendrigh, 1974) that coupling of two oscillators with different natural periods must result in the combined system assuming an intermediate period. Depending on the amplitude (coupling) coefficients A_E and A_M, and the phases of the phase response curves ϕ_E and ϕ_M, mutual coupling can make the combined system either faster than or slower than either oscillator. Second, the simulations shown in Figs 4 and 5 suggest that splitting may occur in different ways. Changes in the periods τ_E and τ_M with light intensity need not be involved. It also works if A_E and A_M, the magnitudes of the oscillators' influence on each other, are functions of light intensity.

In proposing such a mathematical development, one is led to ask whether the features simulated depend heavily on the particular instructions used. By manipulating the shape of the phase response curves in Fig. 2 one can easily see that stable equilibria only occur when the magnitude of the phase shifts

varies with the phase of the cycles, but on the other hand that widely different shapes of these curves are allowed. While our simulations used sinusoidal response curves to simplify the algorithm, this is not a necessary condition to produce the results obtained. Coupling between oscillators necessarily involves phase shifts, whether abrupt as in our model, or gradual. If these shifts are made small enough (weak coupling) the steady state phase relationship between two oscillators will be gradually attained. Hence, we presume that slow changes in τ and α are properties of any system of two weakly coupled oscillators whatever the nature of their coupling. While the interrelationship of τ and α is a property shared by some one-oscillator models (Wever, 1965), the two-oscillator model seems unique in producing these *long term* history-dependent phenomena, known to occur as after-effects and culminating in the hysteresis involved in splitting of activity rhythms. It has been shown elsewhere (Berde, 1976) that long-lasting non-monotonic changes in τ as observed in experiments (Eskin, 1971) are not produced by existing single oscillator models.

Two alternative models have been claimed by Pavlidis (1973, pp. 160–170) to account for the splitting phenomenon. One is based on a population of many oscillators, the other on two oscillators, one of which is unilaterally coupled to the other. The main shortcoming of these models is that they do not attempt to explain the gradual nature of the transitions. The gradual approach to the split condition during which two separate entities are clearly identified, while running for many cycles with slightly different periods is an important feature of the splitting process, and is ignored by Pavlidis' description of "frequency doubling". Our simulations using two mutually coupled oscillators uniquely predict the essential aspects of the gradual approach to the split condition known from real experiments. This model does not pertain to the mechanism or organization of the oscillators, it only suggests that the system behaves as though two separate entities are involved. Each of these oscillators may of course have a complex substructure, such as a collection of tightly coupled oscillating neurons. The model does not pinpoint which of the parameters of the two oscillators is affected by light. Light may have differential influences either on the spontaneous frequency or on the coupling strength (amplitude) of the oscillators. Splitting and refusion of two components as well as long-term changes in frequency may be a result of both.

We close with some speculations concerning the nature of the two oscillators involved. It has become clear during our work exploring large arrays of parameter values, that coupling between the oscillators generally must be weak to produce long-lasting after-effects. With strong coupling (A_E and A_M large), the system moves fast to its stable equilibrium. However, with weak

coupling, the detuning (D) of the two oscillators must also be small to retain synchrony among them, since this requires that:

$$|D| \leq A_E + A_M.$$

This implies that each of the two oscillators is by itself a precise and stable circadian pacemaker. The stability of mammalian activity rhythms should not be attributed merely to the coupling between the two. The analysis in Fig. 2 shows that two stable equilibria occur only when detuning is small and the strength of coupling is nearly equal in both directions. By increasing the amplitude of one of the phase reset curves in Fig. 2(d), one of the two stable equilibria soon disappears. Thus slight dominance of one of the oscillators over the other quickly removes the possibility of a reversal of phase relationships. On the one hand, it is then not surprising that splitting has only been observed in a minority of the species investigated in constant conditions. On the other hand, in those species where splitting does occur this suggests that the strength or amplitude of the two oscillating processes are of comparable magnitude.

These lines of thought lead us to speculate that, if two oscillators are involved in rodent circadian clocks, they may be quite similar systems, each in itself fully capable of functioning as a true circadian pacemaker, but loosely coupled to each other. Is bilateral symmetry in the organization of the nervous system involved? Evidence is accumulating that the pacemaker in rodent circadian rhythms is located in the two suprachiasmatic nuclei (SCN) of the hypothalamus (Stephan & Zucker, 1972; Moore & Eichler, 1972; Stetson & Watson-Whitmyre, 1976). Lesions of the SCN have been found to produce splitting-like phenomena in the activity rhythms of hamsters (Rusak & Zucker, 1975) and, when administered unilaterally in mice, to induce consistent changes in τ (van Buskirk, pers. comm.). While such phenomena would be expected to result from a change in the parameters of coupling between two oscillators, their interpretation clearly awaits more knowledge of the anatomical distribution and physiological detail of circadian pacemakers.

Functional considerations on such a two-oscillator system, regardless of concrete mechanism, have been explicated by Pittendrigh & Daan (1976*c*). A double system with slight differences in the effect of light on the two oscillators would provide a means to adjust the internal temporal program to the seasonally changing characteristics of the light-dark cycle as well as to measure daylength. It would further be apt to produce the bimodal activity pattern widely distributed among animals (Aschoff, 1962). If the two oscillators supposedly involved in these functions were indeed anatomically distinct one might hope to study their organization and interaction.

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